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Award Number: DAMD17-00-1-0431

TITLE: Breast Cancer Susceptibility Genes in High Risk Women

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REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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20030109 100

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> July 2002	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Jul 01 - 30 Jun 02)	
<b>4. TITLE AND SUBTITLE</b> Breast Cancer Susceptibility Genes in High Risk Women			<b>5. FUNDING NUMBERS</b> DAMD17-00-1-0431	
<b>6. AUTHOR(S)</b> Ann S. Hamilton, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> University of Southern California Los Angeles, California 90033  E-Mail: ahamilt@usc.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)</b> A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer. The genetic factors responsible are largely unknown, although the autosomal dominant, relatively high penetrant genes BRCA1/2 may account for 3%. It has been hypothesized that susceptibility genes of lower penetrance may also affect breast cancer risk, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen. Previous studies of these susceptibility genes have not been conducted with women with high familial risk. This study is being conducted with identical twins with differing genetic risks (i.e. concordant for breast cancer pairs vs. discordant pairs) as well as unaffected controls. We have chosen to focus on those genes related to estrogen metabolism and carcinogen metabolism. In the estrogen metabolism pathway, polymorphisms have been described related to the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 gene. Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. We will compare the frequency of selected polymorphisms in these genes in 200 breast cancer concordant, 200 discordant, and 200 control women. We currently have tissue or buccal smears and informed consents from 85 concordant, 108 discordant, and 101 control women. Preliminary laboratory analyses of the CYP17 gene indicate that this gene is not associated with a high genetic risk. underway.				
<b>14. SUBJECT TERMS</b> breast cancer, CYP17, CYP19, COMT, CYP1A1, HSD17B1, GSTM1, GSTP1, twins, estrogen metabolism, carcinogen metabolism, genetics				<b>15. NUMBER OF PAGES</b> 17
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

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#### **4) INTRODUCTION**

A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer(1), and this is true for postmenopausal as well as the premenopausal cases, among which the autosomal dominant, relatively high penetrant genes BRCA1 and BRCA2 are most prominent(2). It has been hypothesized that susceptibility genes of lower penetrance are more prevalent than among the latter, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen (3), the common factor underlying most known predictors of breast cancer risk (4) (5) (6). Recent reviews have identified several candidate genes (7) (8) (9). We have chosen to focus on those genes related to estrogen metabolism and carcinogen metabolism.

In the estrogen metabolism pathway, four genetic polymorphisms have been described related to the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 (or also called the EDH17B2) gene. For example, a polymorphism (called A2) on the CYP17 gene has recently been linked to higher endogenous estrogen levels and an earlier age at menarche (10). The same polymorphism was linked to increased risk of aggressive breast cancer, although one attempt to confirm this finding was unsuccessful(11). Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. These studies, however, have not been conducted with women known to be at high familial risk, where the prevalence of the polymorphism may be expected to be higher, if it is associated with the development of breast cancer. This study proposes to take advantage of a unique subset of very high risk women in whom cumulative exposure to endogenous estrogen may play an especially important role in breast cancer etiology.

The identification of families to study these inherited genetic factors is more difficult because of the anticipated lower penetrance of the candidate genes and occurrence of more sporadic cases, especially among older women. The International Twin Study includes both breast cancer concordant and discordant identical twin pairs. The concordant MZ twin pairs represent families with a very high familial risk of breast cancer, while the MZ discordant twins are likely to represent non-heritable cancer. We plan to obtain DNA from subsets of these pairs as well as from control women without breast cancer (and without a family history of breast cancer) and to test for the genetic polymorphisms specified to determine if any are differentially associated with cases from twins with a high likelihood of heritable breast cancer (i.e. those from identical concordant pairs). This study should provide important clues regarding other genetic factors that may be associated with breast cancer etiology. Initial work on the project and the CYP17 laboratory work was funded under a grant from the California Breast Cancer Research Project (CA-BCRP).

#### **5) BODY**

Technical Objectives and Work Accomplished in year 2:

*Task 1: To complete follow-up of female identical twin pairs with breast cancer (Months 1-18)*

1. Continue follow-up begun under CA-BCRP grant
2. Hire Programmer, set up tracking database

3. *Continue to mail follow-up forms with return envelope to last known address of twins. Enter data from responses.*
4. *Submit nonrespondent names to National Death Index.*
5. *Submit names of nonrespondent twins not known to be deceased to TRW/ Experian to obtain updated addresses. Resend follow-up forms.*
6. *Continue follow-up by phone calls, internet searches, and contact with relatives.*

It was previously reported that a data file was created from the International Twin Registry that selected all of the identical female twin pairs in which one or both members had been diagnosed with breast cancer. In total there are 1,491 identical pairs in this database and 1,199 of them were initially classified as discordant pairs, 263 as concordant, and 29 of uncertain concordance. A follow-up form was sent to all living members of all of the discordant pairs, and new breast cancers have been reported in the previously healthy twin of 62 of these pairs. Thus as a result of this information, there are now 338 concordant pairs and 1,153 discordant pairs. Follow-up efforts have consisted of mailing 1,883 follow-up forms to living twins in these pairs, and 1,029 have been returned completed. 260 were returned by the post office and 478 were not returned by either the twin or the post office. Tracing efforts were implemented to locate the nonrespondents. Follow-up of all nonrespondents will continue using the National Death Index. A file to the NDI has been submitted and results are pending. (This component was funded under the CA-BCRP grant).

*Task 2: Identify new breast cancers and obtain medical record documentation and tissue blocks. (Months 6-20)*

1. *When new breast cancer is identified, obtain medical consent form from twin or next of kin, and request records and tissue blocks from hospital*
2. *Follow-up requests with hospitals*

The goal of the study is to obtain genomic DNA from at least one member of 200 of the concordant pairs, from the case in 200 of the discordant pairs, and from 200 control women without a personal or family history of breast cancer. From a previous study, tissue blocks have been obtained from some of the breast cancer pairs (concordant and discordant). As a result of the follow-up effort, we have identified 62 previously discordant pairs in whom the unaffected member has developed breast cancer. Thus the number of concordant and discordant pairs has been adjusted to reflect the current status.

To participate in the study, the eligible participants are sent a letter describing the study along with the informed consent documents. Our study manager then calls the twin to go over the informed consent with her over the telephone. Then if she agrees to participate and donate the required tissue to the study, she then signs the informed consent form and mails it back to us. .

As of this time (7/19/02) the current numbers of MZ twins (and controls) in each subset with tissue and signed consent forms is the following:

	Concordant	Discordant	Controls
Number identified	233	1023	126
Buccal kit sent	19	1	122
Tissue available (and has not refused to signed informed consent)*	136	187	
Buccal smear received	13		101
DOD consent signed and tissue/buccal smear available	85	108	101
Goal	200	200	200
Additional cases/controls needed to reach Goal	115	92	99
Additional cases who could be sent buccal smear kit	72	835	
Cases with tissue blocks obtained previously who are being contacted to sign the informed consent for this study	64	79	
Potential subjects	136	914	

\*so far we have had 30 cases refuse to participate in this study. Reasons included not interested, and too busy as well as the language that the DOD requires us to include in the informed consent regarding 'POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH'.

We are continuing the process of obtaining the DOD consent form from the concordant and discordant cases with tumor tissue available. In order to reach our sample size goal of 200 in each category we will continue to pursue the 136 concordant pairs, especially the 64 for whom we already have tissue. We have ample discordant pairs to meet our goal and will focus on the 79 for whom we already have tissue. We are identifying controls at the same time as we recruit the cases and anticipate no problems in reaching our goal of 200 controls.

*Task 3: Obtain buccal smears from living member of case pairs when blocks not available (Months 1-20)*

1. *If tissue blocks are no longer available from either member of the case pairs and there is a living twin, send letter to obtain buccal smear.*
2. *Send buccal smear kit and return mailing supplies and postage to these individuals.*

The procedures for obtaining buccal smears have been developed and kits have been assembled for this purpose. We are using Epicentre Technologies Master Amp Buccal Swab Brush. Two brushes are being sent to the selected cases (and controls) and they are asked to use one for each cheek. Once the swabs are returned to us they are being kept frozen until the laboratory analyses are done. To date we have sent the swabs to 19 concordant pairs (and received 13 back) and to 1 discordant pair.

*Task 3: Identify 200 control women and obtain buccal smear and risk factor questionnaire from each of them*  
(Months 1-20)

1. *Contact case pairs to obtain listing of unrelated breast cancer free potential control women selected from sisters-in-laws and friends.*
2. *Randomly select a women from this list and mail introductory letter.*
3. *Obtain buccal smear and risk factor questionnaire from each control woman through the mail.*

We have developed the protocol for selecting controls and this is working well. To date we have identified 126 controls and have sent the kits to 122 of them, and 101 have been returned.

*Task 4: Laboratory analysis of DNA from tissue and buccal smears to identify polymorphisms in the specified breast susceptibility candidate genes*  
(Months 1-24)

1. *Finish CYP-17 analysis at Dr. Dubeau's Laboratory.*
2. *Extract additional DNA as necessary for the additional genetic tests.*
3. *Do additional tests for CYP19, COMT, HSD17B1, GSTM1, GSTP1, and CYP1A1.*
4. *Receive results and enter data into database.*
5. *Store tissue for future genetic studies.*

Dr. Dubeau's laboratory has completed the CYP17 assay for 70 concordant and 97 discordant pairs.. We have also had the DNA extracted from the first 96 of the controls who provided the buccal sample, although the CYP17 results are not completed as yet. In addition, since it was difficult for some samples to extract the DNA from the archived tissue, Dr. Dubeau has repeated the assay using linear PCR for several of the samples to assure reliability of the assay.

*Task 4 Data analysis (Months 18-32)*

1. *Link data on genetic factors to other information from twins and controls including risk factor information and other tumor related information when available (e.g. ER positivity)*

2. *Complete analyses of data to determine relationship of the specified polymorphisms to breast cancer susceptibility.*
3. *Submit papers and reports.*

Table 2 shows the results of the CYP17 on the cases which have been completed. Controls from literature sources are provided for comparison, since our controls have not been assayed yet. The A2 allele has been associated with higher endogenous estrogen and would be expected to be more prevalent in the concordant pairs, if it was a polymorphism associated with a higher genetic risk of breast cancer in this twin subset. However, the proportion with the A2 allele (including those with A1/A2 or A2/A2) was lower in the concordant pairs (55.7%) than in the discordant pairs (72.2%). The controls from literature sources had 64-65% with either of these allele combinations. Thus, the preliminary results do not support this polymorphism in the CYP17 gene as being related to an increased genetic risk in these twins.

Table 2: Percent distribution of CYP17 genotype by type of breast cancer twin pair compared to controls from literature sources (Preliminary data)

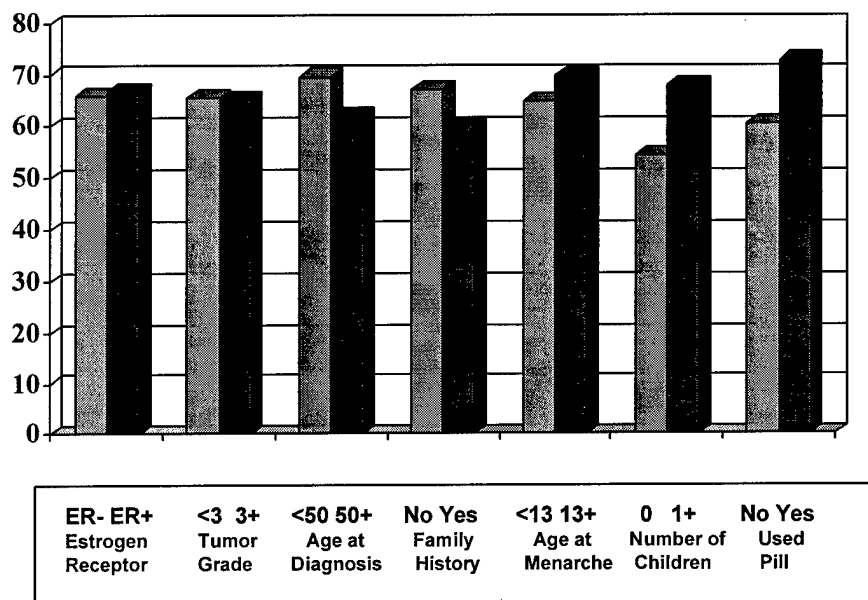
CYP17 Genotype	Type of Breast Cancer Twin Pair*		Controls from Literature Sources	
	Discordant N (%)	Concordant N (%)	Nurses Health Study <sup>a</sup> N (%)	Multi-Ethnic Cohort Study <sup>b</sup> N (%)
A1/A1	27 (27.8)	31 (44.3)	217 (35.1)	222 (36.1)
A1/A2	63 (65.0)	31 (44.3)	307 (49.7)	287 (46.7)
A2/A2	7 (7.2)	8 (11.4)	94 (15.2)	106 (17.2)
Total	97 (100.0)	70 (100.0)	618 (100.0)	615 (100.0)
%A1/A2+ A2/A2	70 (72.2)	39 (55.7)	401 (64.9)	393 (63.9)

\*  $\chi^2$   $p < .05$  for difference between distributions of discordant and concordant pairs

<sup>a</sup> Haiman, C., Hankinson, S., Spiegelman, D., Colditz, G., Willett, W., Speizer, F., Kelsey, K., and Hunter, D. The relationship between a polymorphism in CYP17 with plasma hormone levels and breast cancer.

<sup>b</sup> Feigelson, H., McKean-Cowdin, R., Coetzee, G., Stram, D., Kolonel, L., and Henderson, B., Building a multigenic model of breast cancer susceptibility: CYP17 and HSD17B1 are two important candidates. Cancer Research 61:785-789, 2001.

Figure 1: Percent with CYP17 A1/A2 or A2/A2 allele by selected tumor characteristics and risk factors for breast cancer (based on 167 cases from discordant and concordant pairs)



We also compared the tumor characteristics of cases with an A2 allele to those cases without an A2 allele and found no significant differences (Figure 1).

#### 6) Key Research Accomplishments

- Preliminary data on CYP17 results obtained
- Over 100 controls have been identified and have provided buccal smears.
- DNA has been extracted from buccal smear samples.
- Preliminary analyses do not support a predisposing genetic risk of breast cancer for carriers of the A2 allele, nor is it linked to specific tumor characteristics.

#### 7) Reportable Outcomes

- We have obtained DNA samples and consents from 85 concordant cases, 108 discordant cases and 101 controls. The goal is to obtain 200 in each group by the completion of the study.
- Laboratory work on the CYP17 gene has been completed for 167 cases.
- No increased genetic risk was found for the A2 allele in the CYP17 gene.

#### 8) Conclusions

- The only significant difference found so far is that there was a higher proportion of twins

with the A2 allele (primarily in the A1/A2 group) among the breast cancer discordant twin pairs than among the breast cancer concordant pairs.

- The distribution of alleles in the concordant pairs was similar to that found for control groups in other studies.
- These findings are opposite to that which might have been expected since the concordant pairs are more likely to represent genetically susceptible cases. This may imply that the CYP17 gene is not associated with a predisposing genetic risk in these twins.
- No significant differences in the CYP17 allele frequency were found by tumor characteristics or breast cancer risk factors.

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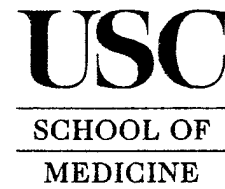
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## **10) APPENDICES**

Currently approved informed consent documents

Please initial each page: \_\_\_\_\_

USC Norris Comprehensive Cancer Center  
Keck School of Medicine  
Department of Preventive Medicine  
1441 Eastlake Ave., Rm. 3427, MC9175  
Los Angeles, CA 90089-9175



**1. Description of the Medical Research for which your participation is requested**  
(Please sign and return one copy and keep the second copy for your records)

**TITLE OF PROJECT:** Breast Cancer Susceptibility Genes in High Risk Women

**PRINCIPAL INVESTIGATOR:** Ann Hamilton, Ph.D. (323)-865-0434

**DEPARTMENT:** Department of Preventive Medicine, Keck School of Medicine at the University of Southern California, 1441 Eastlake Ave, Los Angeles, CA 90089.

**24-HOUR TELEPHONE NUMBER:** Toll free number: 800-421-9631

**PURPOSE OF THE STUDY:** You are invited to participate in a research study of genetic factors that may be related to the development of breast cancer. You are invited to be a participant because you are a member of a twin pair in which one or both of you have been diagnosed with breast cancer.

The genes that we are studying include those that control the amount of estrogen produced in the body and those that control the removal of cancer causing substances from the body. (We will not be testing for BRCA1 and BRCA2, the rare genes that are known to increase risk of breast cancer). To test for these genes, we will use a sample of your DNA that will be obtained from tissue that was preserved at the time of your breast cancer surgery.

**PROCEDURES:** We will send you a letter explaining this new study and ask you to sign a release form giving us your permission to contact the hospital and request the tissue blocks, if we have not done so already for a previous study. A few thin slices will be cut from the blocks. We will ask the hospital if they require that the blocks be returned and, if they do, the blocks will be returned. Otherwise, we will maintain the blocks in storage.

Laboratory analyses of the tissue block samples to determine the presence of genetic factors will be done with personal identifiers removed. Thus, it will not be possible to link results with specific individuals.

We may also ask you for your permission to contact a sister-in-law or friend without cancer to participate in the study, after you have discussed the study with them

**RISKS:** There are no physical risks. There is a small risk of loss of confidentiality, however all records are secured and kept confidential. While genetic testing will be done for this study, the results cannot be linked to a specific individual, thus risks related to genetic testing will not apply. For the genes being tested, the risk associated with development of breast cancer is unknown at this time.

Please initial each page: \_\_\_\_\_

**BENEFITS:** You may receive no direct benefit from your participation in this study. However, your participation may help us learn if certain genetic factors may be related to development of breast cancer.

**ALTERNATIVES TO PARTICIPATION:** An alternative would be not to participate in this study.

**CONFIDENTIALITY STATEMENT:** We will maintain the confidentiality of your medical records to the extent permitted by law. Data are kept in locked file cabinets and genetic information is not linked to personal identifying information. The information from this study may be published in scientific journals or presented at scientific meetings. Published results will only consist of numbers of persons arranged in categories. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

**OFFER TO ANSWER QUESTIONS:** You may contact the Principal Investigator at the number listed above if you have any questions about the study. If you have any questions regarding your rights as a research subject, you may contact the Institutional Review Board office (IRB) at 323-223-2340.

**COMPENSATION:** The availability and quality of your medical care will not be affected by your participation or refusal to participate. We can provide no compensation for your participation in this study.

**VOLUNTARY PARTICIPATION AND WITHDRAWAL:** Your participation in this research study is voluntary. Your decision whether or not to participate will not interfere with your right to health care or other services to which you are otherwise entitled. If you do decide to participate, you are free to withdraw your consent and discontinue participation at any time.

**POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH AND DONATION OF TISSUE SAMPLE.** The following paragraph is included at the request of the funding agency for the study, the Department of Defense. We feel that the possibility of a commercial application is remote.

During this study, you will be asked to provide tissue block samples. These samples will be used for studying genes that may be related to breast cancer and may also be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. Should your donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and may take action to patent and license the product. The Keck School of Medicine at the University of Southern California does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have.

Please initial each page: \_\_\_\_\_

As a participant in this study called 'Breast Cancer Susceptibility Genes in High Risk Women', I voluntarily donate any and all tissue samples to the Keck School of Medicine at the University of Southern California. I have read the above paragraph and agree that should my donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and it is possible that it will be patented and licensed by the Keck School of Medicine at the University of Southern California. I will not receive any compensation for this.

*Please write your initials in the box to indicate your agreement. Thank you.*

**INFORMATION ABOUT TISSUE SAMPLES COLLECTED AS PART OF THIS**

**RESEARCH:** The tissue used in this study is from tissue blocks already collected at the time of breast cancer surgery. The amount of tissue needed to do the genetic tests is very small, amounting to a few thin slices on a slide. The use of this tissue will reduce, but only very slightly, the amount of tissue remaining to conduct future studies or diagnostic tests.

In the future it may be important to test this tissue for newly discovered genetic factors that may be related to the development of breast cancer. We will be storing some of the tissue for this purpose in the future. Please initial below if you give your consent for additional genetic testing to be done in on these samples

I give consent for additional genetic testing related to breast cancer to be done in the future on my stored tissue samples.

*Please write your initials in the box to indicate your agreement. Thank you.*

**CONSENT PROCEDURE:** Initial contact with participants in this study is made by a letter describing the study purpose and procedures. Participants are called and this document is discussed with them and they are asked to sign and return a copy of this document.

**CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:**

1. The nature and purpose of the study.
2. The procedures in the study and any drug or device to be used.
3. Discomforts and risks to be expected from the study.
4. Benefits to be expected from the study.
5. Alternative procedures, drugs or devices that might be helpful and their risks and benefits.
6. Availability of medical treatment should complications occur.
7. The opportunity to ask questions about the study or the procedure.
8. The opportunity to withdraw at any time without affecting your future care at this institution.
9. A copy of the written consent form for the study.
10. The opportunity to consent freely to the study without the use of coercion.
11. Statement regarding liability for research-related injury, if applicable.

Please initial each page: \_\_\_\_\_

**AGREEMENT:**

I have read (or someone has read to me) the information provided above. I have been given the opportunity to ask questions and all of my questions have been answered to my satisfaction. My signature below indicates that I have decided to participate having read the information provided above.

Name of Subject	Signature	Date Signed
Street Address	City	State, Zip

Thank you! Please mail the signed copy in the enclosed postage paid envelope or to the address listed below.

International Twin Study  
Attn: Dr. Ann Hamilton  
USC/Norris Comprehensive Cancer Center  
1441 Eastlake Ave., Rm 3427, MC9175  
Los Angeles, CA 90089-9175

**Interviewer's statement and signature**

'I have discussed the above information with the subject and answered all of the subject's questions regarding the study. It is my opinion that the subject understands the risks, benefits, and obligations involved in participation in this project'.

Name of Interviewer	Signature	Date Signed
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Form Valid For Enrollment From
APR 15 2002 To APR 14 2003
Institutional Review Board